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13. ABSTRACT ***(Maximum 200 Words)***

This project will assess the association between the risk of breast cancer and polymorphysms of the androgen (AR) and vitamin D receptor (VDR) genes among subjects in a recently-completed population-based case-control study in Sweden. A total of 3879 cases and 3527 controls took part, providing quesionnaire data regarding use of exogenous hormones and other life style factors. From this study population, breast cancer cases and control women have been randomly selected for genomic DNA analysis. The collection of blood or tissue specimens for DNA has been funded from other sources; this award is for the measurement of the AR and VDR polymorphisms on 300 cases and 300 controls who never used HRT, and a similar number of cases and controls who used HRT for 4 years of more. Information on these polymorphisms will be incorporated into the established subject database, and odds ratios summarizing the associations with breast cancer risk will be computed. In the two first years of the project, the work was organized, and a tracking database for subject recruitment and specimen accrual was built. Recruitment into the molecular epidemiology study is almost complete, and the laboratory analyses will be finished in the forthcomming months. We expect to perform data analysis and reporting of the study results during the next year.

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Introduction

This project will assess the association between the risk of breast cancer and polymorphisms of the androgen (AR) and vitamin D receptor (VDR) genes among subjects in a recently completed population-based case-control study in Sweden. A total of 3879 cases and 3527 controls took part, providing questionnaire data regarding use of exogenous hormones and other life style factors. From this study population, breast cancer cases and control women have been randomly selected for genomic DNA analysis. The collection of blood or tissue specimens for DNA has been funded from other sources; this award is for the measurement of the AR and VDR on 300 cases and 300 controls who never used HRT, and 300 cases and 300 controls who used HRT for 4 years of more. Information on these polymorphisms will be incorporated into the established subject database, and odds ratios summarizing the associations with breast cancer risk will be computed. In the first first two years of the project, the administrative arrangements for the work were established, and a tracking database for subject recruitment and specimen accrual built. Recruitment into the molecular epidemiology study and the laboratory analysis will be completed in the forthcoming months. We expect to perform data analysis and reporting of the study results during the next year.

Progress to Date

Under previous funding, all questionnaire data have been obtained and organized, and several manuscripts dealing with the questionnaire data have been published (see previous report).

Administrative Preliminaries:

The grantee organization, Dartmouth College, is maintaining a subcontract with the Karolinska Institutet for the collaborative work described in our proposal. The Karolinska Institutet has an on-going relationship with investigators at Uppsala University in Uppsala, Sweden, where the molecular analysis are being conducted.

Recontacting study subjects to obtain germline DNA for analyses:

This work, still in progress, has been funded by other awards from the National Institutes of Health and from the Army Medical Research and Materiel Command Breast Cancer Research Program.

We aimed to obtain blood or tissue sample from 1798 breast cancer patients, selected from the 3879 cases initially enrolled in the study. This Army award will support the analysis of a subgroup of these subjects (600 cases); lab work for the others will be supported by other sources (NIH and another USAMRMC award).

We have been successful in obtaining germline DNA in 95.3% (1713) of the selected cases. 1321 (73.5% of the 1798 sampled) donated a blood sample, and an additional 392 cases (21.8% of 1798) who had died or who refused to donate a blood sample allowed us to use stored surgery specimens. Sixty-seven patients (3.7% of 1798) refused to participate in this phase of the study, and we were unable to trace 16 others. We are still trying to obtain a blood sample from 1 remaining case patient.

We aimed to obtain blood or tissue samples from 1564 control women (without previous breast breast cancer), selected from the 3527 control women initially enrolled in the study. As for cases, this number excedes the total that will be analysed using resources from this award (600 controls). We have obtained blood samples from 1156 control women (74.0%). 384 (24.6%) control subjects declined to participate, and 14 (0.9%) could not be located. We are hoping to obtain blood samples from 8 additional controls.

Laboratory analysis

After being entered into the administrative tracking system (see last year's report), all blood/tissue speciments are sent to the laboratory in Uppsala, Sweden. DNA is being extracted, and the assays being performed. We expect to complete laboratory analyses for all participants by the end of calendar year 2000.

Analysis and Reporting

Statistical analysis and writing of scientific manuscripts and reports will be done in year 2001.

Training

The project has played a role in the training of two investigators. The data generated will be part of the Ph.D. thesis of Ms. Sara Wedren, and the work is also part of the activities for the post-doctoral fellowship of Dr. Elisabete Weiderpass.

Appendix I. Key Research Accomplishments

- completion of organizational prerequisites
- construction of a detailed administrative database
- successful recruitment of subjects to the molecular epidemiology study
- completion of laboratory analyses for the majority of subjects

Appendix II. Reportable outcomes

a) Manuscripts, abstracts, presentations:

The post-doctoral student working in the project (Elisabete Weiderpass, Karolinska Institutet, Stockholm) presented two posters describing the project at the 'Era of Hope' conference (Atlanta, Georgia, June, 2000). Copies of the abstracts are attached.

b) Patents and licenses applied for and/or issue

None

c) Degrees obtained that are supported by this award:

The Ph.D. thesis work of a graduate student in Cancer Epidemiology, Ms. Sara Wedren (Karolinska Institutet, Stockholm, Sweden), is partially drawn from this project. She is expected to defend her thesis, thus obtaining her Ph.D. degree, at the end of 2001 at the beginning of 2002.

d) Development of cell lines, tissue or serum repositories:

a biological bank containing DNA samples from breast cancer patients and control women is being created and will be mantained using finantial resources obtained elsewhere. The creation and maintenance of the biological bank follows the Swedish law for storage of biological samples for scientific purposals.

e) Informatics such as databases and animal models, etc:

- An administrative database containing information about study subjects has been created at the Karolinska Institutet;
- A database containing questionnaire information from breast cancer case patients and control women has been created at the Karolinska Institutet, and the questionnaire information has been already checked out and corrected for typing errors and logical inconsistencies.
- Programs (using SAS programs) allowing calculation of time of use of different sorts of postmenopausal hormones have been developed and tested at the Karolinska Institutet.
- Databases containing results from the laboratory analyses have been created in Uppsala, Sweden, where the laboratory analysis is being performed. These files will be transferred to the Karolinska Institutet at the completion of the laboratory analysis (expected date: end of January 2001).

f) Funding applied for based on work supported by this award:

A post-doctoral fellowship in Cancer Epidemiology was granted from the Swedish Cancer Society to Dr. Elisabete Weiderpass (Karolinska Institutet, Stockholm, Sweden) to work in this project. This fellowship covers partially her salary from January to December 2000.

g) Employment or research opportunities applied for and/or received based on experience/trainning supported by this award:

The graduate student working on the project, Ms. Sara Wedren, obtained a training fellowship from the Karolinska Hospital, to complete her medical training. She qualified for this fellowship program in part because of her experience in this project.

The post-doctoral fellow working on the project, Dr. Elisabete Weiderpass, has being appointed Docent in Cancer Epidemiology at the Karolinska Institutet, Sweden, in September 2000. She qualified for this position in part because of the experience she acquired working in the project.

HORMONE RECEPTOR GENETIC VARIATION AND BREAST CANCER

Elisabete Weiderpass[a], Sara Wedrén[a], Håkan Melhus[b], Ingemar Persson[a], Hans-Olov Adami[a], John Baron[a, c].

[a]Dept of Medical Epidemiology, Karolinska Institutet, Stockholm, Sweden; [b]Dept of Medicine, Uppsala University, Sweden; [c]Dartmouth Medical School, Lebanon, NH, USA.

Much of the variation in breast cancer occurence is not explained by known risk factors for the disease. Estrogen-, androgen-, and vitamin D receptors (ER, AR and VDR) have important effects in tissues responsive to sex hormones, and therefore may be of etiologic importance. Variation between individuals in the receptor genes may cause varying sensitivity to steroid hormones and explain why some women develop breast cancer and some do not. Steroid hormone receptor gene variants have been associated with breast cancer in some studies, but these studies are relatively small and attention to covarying factors that may modify or confound the association has been limited. Our objective is to test the hypothesis that individual variation in the sensitivity to steroid hormones affects a woman's risk of breast cancer.

In a nation-wide case-control study, we are collecting DNA from 1800 post-menopausal women with breast cancer and 1700 post-menopausal women without the disease serving as control subjects. We are analyzing known genetic variants of the ER, AR, and VDR gene. The frequency of the genetic variants will be compared among cases and controls, taking into account other risk factors for breast cancer that may be associated with, or may modify, genetic effects. Particular attention will be paid to the role of hormone replacement therapy. Our research aims to clarify the importance of the steroid hormone receptors in the etiology of breast cancer.

The U.S. Army Material and Medical Command under DAMD17-97-1-7322 and DAMD17-98-1-8301 supports this work.

HORMONE RECEPTOR GENE POLYMORPHISM AND BREAST CANCER

Elisabete Weiderpass[a], Sara Wedrén[a], Håkan Melhus[b], Ingemar Persson[a], Hans-Olov Adami[a], John Baron[a, c].

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Most of the variation in breast cancer occurrence is not explained by known risk factors for the disease. Estrogen-, androgen-, and vitamin D receptors (ER, AR and VDR) have important effects in tissues responsive to sex hormones, and therefore may be of etiologic importance. Variation between individuals in the receptor genes may cause varying sensitivity to steroid hormones and explain why some women develop breast cancer and some do not. Steroid hormone receptor gene variants have been associated with breast cancer in some studies but these studies have been relatively small and attention to covarying factors that may modify or confound the associations has been limited. Our objective is to test the hypothesis that individual variation in the sensitivity to steroid hormones affects a woman's risk of breast cancer.

We are collecting germline DNA from 1800 women with breast cancer and 1700 women without the disease. All subjects are postmenopausal and have been randomly chosen among the participants in an earlier population-based case-control study where information about known and suspected risk factors was collected via an extensive questionnaire. We are analyzing the XbaI, PvuII and TA_n polymorphisms of the ER gene, the CAG and GGN polymorphisms of the AR gene, and the poly A polymorphism in the VDR gene. The association of the genetic variants with breast cancer risk will be analyzed using logistic regression models, taking into account possible confounders. In stratified analyses particular attention will be paid to the putative role of postmenopausal hormone replacement therapy as an effect measure modifier.

We are currently concluding the collection of biological material from the study subjects. To date the participation rate is 90 % and 80% among cases and controls, respectively. Approximately half of the samples have been analyzed. All analyses are expected to be complete by the end of year 2000. Our research aims to clarify the importance of the steroid hormone receptors in the etiology of breast cancer.

The U.S. Army Material and Medical Command under DAMD17-97-1-7322 and DAMD17-98-1-8301 supports this work.